Demonstration of Antigenic Sites in Glomeruli of Patients with Acute Poststreptococcal Glomerulonephritis by Immunofluorescein and Immunoferritin Technics

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The presence and localization of antigenic sites in glomeruli of 14 patients with acute poststreptococcal glomerulonephritis (AGN) were studied by immunofluorescein and immunoferritin technics. Labeled IgG fractions from the same patients were used for the identification of antigenic sites. The staining capacity of these IgG fractions depended on the time when sera were obtained. Staining was minimal during the first week, and increased up to the fourth or fifth week. Glomeruli, however, stained only when renal tissue was obtained during the early phase of the disease. Precise localization of antigenic sites was determined with ferritin-conjugated patients' IgG. Segmental deposition of ferritin was observed in the mesangial matrix and on the endothelial side of the glomerular basement membrane. Subepithelial electron-dense deposits contained no or very few ferritin particles. In contrast, ferritin-conjugated antihuman IgG was distributed diffusely in the mesangial matrix, on the endothelial side of the basement membrane and in subepithelial deposits. These findings suggest that, during the early stage of acute poststreptococcal glomerulonephritis, free antigen is present in the glomeruli of patients with this disease (Am J Pathol 70:131–150, 1972).

IT IS GENERALLY ACCEPTED that an antigen-antibody reaction plays a role in the pathogenesis of acute poststreptococcal glomerulonephritis; the exact nature of the antigen, however, is still a matter of dispute. Antibodies to streptococcal products are present in the sera of patients with acute glomerulonephritis,[1–3] and several investigators have demonstrated the presence of streptococcal antigen in glomeruli.[4–7] In a previous report,[8] we showed that fluorescein-labeled immunoglobulin G fractions of serum from patients with acute poststreptococcal glomerulonephritis stained parts of the glomerular basement membrane and mesangium of kidney tissue obtained from the same patients in the early phase of the disease.

The present report contains further data concerning the precise localization and the rate of appearance and disappearance of antigenic

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sites in the glomeruli of patients with poststreptococcal glomerulonephritis, using immunofluorescein and immunoferritin technics.

Materials and Methods

Percutaneous kidney biopsies were performed on 14 patients (13 children and 1 adult) with acute poststreptococcal glomerulonephritis, between 4 and 32 days after onset of the disease. The diagnosis of acute poststreptococcal glomerulonephritis was established by the presence of proteinuria, hematuria and lowered serum complement activity (CH_{50}). Edema, hypertension and an increasing antistreptolysin O (ASLO) titer was observed in most cases. Renal morphology was compatible with acute glomerulonephritis in all cases.

Renal biopsy specimens from patients with subacute glomerulonephritis, chronic glomerulonephritis, systemic lupus erythematosus, Henoch-Schoenlein purpura nephritis, pure nephrosis and membranous nephropathy were used as controls.

Sera from patients with acute poststreptococcal glomerulonephritis were obtained 1 to 9 weeks after the onset of the disease.

Human sera from normal individuals who had no history of glomerulonephritis, no evidence of recent streptococcal infections, and whose ASLO titers were in the normal range were used as controls.

High titered rabbit antisera against human IgG and the third component (β1C) of complement were prepared in our laboratory.

Preparation of Sera for Fluorescein Labeling

The IgG fractions of the sera to be used were isolated by diethylaminoethyl (DEAE) cellulose column chromatography using 0.02 M phosphate buffer, pH 8.0.[9] The fractions were labeled with fluorescein isothiocyanate (FITC),[10] and the unreacted fluorescein was removed by gel filtration through Sephadex G25. Labeled fractions were adsorbed with fresh homogenized rat liver in 1:1 ratio.

Preparation of Ferritin-Conjugated Protein

Ferritin was purified by recrystallization and reprecipitation from "crude" ferritin (crystallized twice and precipitated once; Nutritional Biochemicals Corporation, Cleveland, Ohio). After dialysis against running cold water and phosphate buffer, the purified cadmium-free ferritin was ultracentrifuged at 100,000g for 2 hours. The pellet was dissolved in a small amount of phosphate buffer and adjusted to a concentration of 65 to 70 mg/ml. The ferritin was passed through a Millipore filter and stored at 4 C.

The purified ferritin was mixed in an ice bath with a mixture of one part 0.05 M phosphate buffer, pH 7.5, and one part 0.3 M borate buffer, pH 9.5, in volumes so that a final concentration of 20 to 25 mg of ferritin in buffer was achieved.

Meta-xylylenediisocyanate (Polysciences, Inc, Rydal, Pa) was added to the mixture in the proportion of 0.1 ml per 100 mg of ferritin. The mixture was stirred in an ice bath on a magnetic stirrer for 45 minutes, and then centrifuged at 4 C and 2000g for 30 minutes. IgG or albumin was added to the supernatant containing the ferritin intermediate in the proportion of one part of protein to four parts of ferritin, by weight. A concentation of 30 mg/ml of protein was used. Fresh borate buffer was added to maintain 0.1 molarity and pH 9.5, and the mixture was stirred gently with a magnetic stirrer for 48 hours at 4 C.

The mixture of ferritin-conjugated, unconjugated protein and unconjugated ferritin was dialyzed against 0.1 M ammonium carbonate and, 24 hours later, against 0.05 M phosphate buffer in the cold overnight and was then centrifuged

Table 1—Clinical and Laboratory Findings in 14 Cases of Acute Poststreptococcal Glomerulonephritis

			Evidence for Streptococcal infection				Urine				
Patient	Age sex	Duration between onset and biopsy (days)	Loci	Latent period (wks)	Culture*	ASLO titer	Protein	RBC	CH_{50}†	BUN (mg/100 ml)	B/P (mmHg)
1	11 F	4	Throat	2½	+	2500	+++	Many	0	16	130/90
2	15 F	4	Throat	1½	—	1250	+++	Many	0.31	32	160/120
3	13 M	5	Throat	2	+	250	++	Many	0.79	10	122/80
4	13 M	5	Throat	3	+	1250	+	Many	0.08	27	142/92
5	12 F	5	Throat	2½	+	2500	+	Many	0.25	19	136/90
6	8 M	6	Throat	½	+	125	++	10–20	0.3	27	170/120
7	13 F	6	Throat	2½	ND	1250	+	Many	0.35	13	150/105
8	6 M	7	Throat	3	+	1250	+++	Many	0.12	18	94/60
9	8 F	9	Skin	5	—	1250	+	Many	0.08	11	140/110
10	10 M	10	Throat	1	—	833	+++	Many	0.4	23	156/90
11	31 M	12	Throat	5	—	1250	++	Many	0.67	27	170/110
12	9 M	28	Throat	1	—	333	+	Many	Normal	23	100/80
13	11 M	30	Skin	2	+	250	Trace	Many	0.27	10	110/70
14	6 F	32	Throat	2	ND	625	+++	Many	ND	12	162/108

* Positive cultures for group A, β-hemolytic streptococci from the infection site.
† Normal level is between 1.2 to 3.0 units in our laboratory.
ND = not done.

three times at 100,000g for 4½ hours each time. After each run the heavier conjugated protein and unconjugated ferritin, which formed a pellet, were resuspended in 0.05 M phosphate buffer at pH 7.5. The pellet obtained after the final run was incubated with a small amount of phosphate buffer at 4 C overnight. The resulting solution was passed through a bacterial Millipore filter and stored at 4 C in a sterile container. The final concentration of ferritin-conjugated protein was 20 to 40 mg/ml.

All FITC and ferritin-labeled globulins were tested on Ouchterlony agar diffusion plates and by immunoelectrophoresis for their activity.

Preparation and Examination of Tissue

Kidney biopsy specimens for light microscopy were fixed in 10% neutral formalin and embedded in paraffin. Sections 2 to 3 μ thick were stained with hematoxylin eosin, periodic acid schiff (PAS) and periodic acid silver methanamine. In a few cases, Epon-embedded tissues were prepared for light microscopy and stained with toluidine blue.

Specimens for immunohistologic study were prepared by immediately embedding the biopsy cylinder in fresh rat liver and freezing it for 3 minutes in an isopentane–dry ice mixture.[12] Sections of 3-μ thickness were cut at −20 C in a cryostat, air dried, stained with FITC-labeled sera for 30 minutes in a moist chamber and washed with 0.01 M phosphate-buffered saline, pH 7.1. They were examined under a Leitz ultraviolet microscope with a 200W Osram B high tension mercury vapor bulb and photographed with a Leitz Orthomat camera using Anscochrome 200 outdoor film.

Specimens for electron microscopy were fixed in 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Epon. Ultrathin sections were prepared on a Reichert II microtome. Sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11A electron microscope.

Portions of the biopsy specimens were fixed for 30 minutes at 4 C in 5% phosphate-buffered formalin at pH 7.2, then quick-frozen in an isopentane–dry ice mixture and cut at a thickness of 50 to 100 μ in a cryostat.

The sections were washed with 0.01 M phosphate buffer at pH 7.5 in the cold, immersed for 30 minutes at room temperature in the ferritin-conjugated protein, and thereafter washed three times (for 10 minutes each time) with phosphate buffer in the cold.

The resulting pellets were prefixed in a 2% glutaraldehyde in 0.1 M sodium cacodylate at pH 7.2 to 7.5 for 30 minutes at 4 C, washed with phosphate buffer, then postfixed in osmium tetroxide for 1 hour, dehydrated in graded dilutions of ethanol and embedded in Epon. Sections were stained with uranyl acetate.

Results

The major clinical and laboratory findings of 14 patients with acute poststreptococcal glomerulonephritis are presented in Table 1.

Light Microscopy

The glomerular lesions of all 14 patients were typical of acute glomerulonephritis but varied in severity (Table 2). The lesions observed were graded as follows:

+AGN = focal cellular proliferation and increased mesangial matrix.

Table 2—Renal Morphology in 14 Cases of Acute Poststreptococcal Glomerulonephritis

		Immunofluorescent microscopy (IgG and C3)†			Electron microscopy			
Patient	Light microscopy*	GBM	MES	Granularity	Cellular proliferation	Increased mesangial matrix	Subepithelial deposits	Intra-GBM deposit
1	+++ AGN	+++	—	Fine	+++	++	++	+
2	++ AGN	+++	—	Fine	++	+	—	—
3	+++ AGN	+++	—	Fine	+++	++	++	+
4	+++ AGN	+++	—	Fine	++	++	—	++
5	++ AGN	+++	—	Fine	++	++	+	+
6	++ AGN	+++	—	Fine	+++	++	+	++
7	++ AGN	+++	—	Fine	++	++	++	+
8	+++ AGN	+++	—	Fine	++	++	+++	+
9	+++ AGN	+++	+	Fine & coarse	++	++	+	++
10	++ AGN	+++	+	Fine & coarse	++	+	—	+
11	++ AGN	+++	+	Fine & coarse	++	++	+	++
12	+ AGN	+++	+	Fine & coarse	+	++	+	+
13	+ AGN	++	++	Coarse	+	+++	—	+
14	+ AGN	++	++	Coarse	+	++	+	+

* +AGN = Focal cellular proliferation and increased mesangial matrix; ++AGN = Diffuse cellular proliferation and increased mesangial matrix plus significant polymorphonuclear leucocyte infiltration; +++AGN = ++AGN plus generalized glomerular swelling.

† +++GBM, —MES = Diffuse intense staining of glomerular basement membrane with no mesangial involvement; +++GBM, +MES = Diffuse intense staining of glomerular basement membrane with moderate mesangial involvement; ++GBM, ++MES = Glomerular basement membrane and mesangium were equally stained.

++AGN = diffuse cellular proliferation and increased mesangial matrix as well as significant polymorphonuclear leukocyte infiltration.

+++AGN = generalized glomerular swelling with marked polymorphonuclear leukocyte infiltration, diffuse cellular proliferation, and increased mesangial matrix.

Three patients whose renal biopsies were obtained 28, 30 and 32 days after onset of the disease showed glomerular lesions of +AGN. Six patients whose renal biopsies were obtained up to twelve days after onset had lesions classified as ++AGN, and 5 patients whose renal tissues were obtained up to 9 days after onset were found to have +++AGN grade lesions.

Electron microscopy

In all patients, proliferation of endothelial and mesangial cells was the prominent feature. Polymorphonuclear cell infiltration and narrowing of the capillary lumina were observed in early biopsies. Subepithelial electron-dense deposits were found in 8 of 14 patients, and intrabasement membrane and intramesangial deposits were seen in 13.

Immunohistology

Glomerular Deposition of FITC-Labeled Antihuman IgG and C3

Deposition of IgG and C3 in a granular pattern was demonstrated in the glomeruli of all patients (Table 2). Three types of staining patterns were observed and classified as follows:

+++GBM, −MES = staining of basement membranes in diffuse fine granular fashion without mesangial involvement.

+++GBM, +MES = staining predominantly of the glomerular basement membrane in a diffuse fine and coarse granular fashion with moderate mesangial involvement.

++GBM, ++MES = staining of both glomerular basement membrane and mesangium in a coarse granular fashion.

Patients 1–8 showed the first type of staining. Their renal biopsies were obtained within 7 days after onset. The second type was seen in 4 patients (9–12). Kidney biopsies were obtained in this group 9 to 28 days after onset. Two patients (13 and 14) had kidney biopsies taken

30 to 32 days after onset which showed the staining pattern described in the third group.

Glomerular Deposition of FITC-Labeled IgG Obtained from Patients with Acute Poststreptococcal Glomerulonephritis

Sections from kidney biopsies from all 14 patients were treated with FITC-labeled IgG obtained from 8 patients with acute poststreptococcal glomerulonephritis (FITC-IgG-AGN; Table 3).

Eleven renal biopsy specimens (Patients 1–11) showed positive staining with these sera. Staining was diffuse or segmental in a punctate pattern in the area of the glomerular basement membrane, the mesangium and, possibly, the endothelial or mesangial cells (Figures 3 and 4). Sera obtained 2 to 4 weeks after onset of disease usually showed the highest staining capacity.

Three renal biopsy specimens (Patients 12–14) obtained 28 to 32 days after onset of the disease and control specimens did not stain with these sera.

The renal tissues of Patients 1, 4, 5 and 10 were stained with a series of FITC-IgG-AGN sera obtained from Patients 4 and 5. These sera were taken at weekly intervals from 1 to 9 weeks and 1 to 4 weeks, respectively. Staining was most intense and diffuse with sera obtained 2 to 4 weeks after onset.

FITC-labeled IgG from individuals without glomerulonephritis, without history of recent streptococcal infection and with normal ASLO titers that were used as controls did not stain any of the kidney biopsy specimens.

Localization of Ferritin-Conjugated Antihuman IgG

The renal tissues of 5 patients (3, 5, 7, 8 and 9) were treated with ferritin-conjugated antihuman IgG. In each case, ferritin was specifically located in the glomerular basement membranes, predominantly on the endothelial site (Figure 5), in the mesangial matrix (Figure 6) and in subepithelial deposits (Figure 7). Very few ferritin particles were seen in some of the intrabasement membrane deposits and intramesangial electron-dense deposits, as well as in epithelial foot processes and the endothelial cytoplasm.

Localization of Ferritin-Conjugated IgG Obtained from Patients with Acute Poststreptococcal Glomerulonephritis

Renal tissues from all 14 patients were treated with ferritin-conjugated IgG obtained from two patients with acute poststreptococcal glomerulonephritis (Fe-IgG-AGN; Patients 2 and 15). Three to 5 glomeruli

Table 3—Staining of Glomeruli from 14 Patients with Acute Poststreptococcal Glomerulonephritis Using Fluorescein-Labeled IgG Obtained from the Patients*

FITC-IgG-AGN patient sera	Time obtained† (wks)	Patient 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	2	−			+	+									
	3	+			++	+					+	+	−	−	−
	4	±			+	+									
2	2	++	+		+	+							−	−	−
	3	++	+++		++		+	++		++	+	+	−	−	−
3	4		++	+			±	++	+	+	+		−	−	−
4	1	+			+	+					±				
	2	++			++	++					+	+	−	−	−
	3	++			++	+					+				
	4	+			+	+					±				
	5	−			−	−					−				
	7	−			−	−					−				
	9	−			−	−					−				
5	1				±	±									
	2				+	+									
	3				+	+									
	4				++	++									
9	3	±	+++		+	+	±	+		++	+	+	−	−	−
11	3	±			+	±					+	−	−	−	−
15‡	3		+	+++			±	++	+	+					

* Specific staining was rated + to +++: ± = weak and very sparse areas of fluorescence; + = readily identified, but only a small part of staining; ++ to +++ = staining increasing to diffuse localization.

† Time after onset of acute glomerulonephritis when sera were obtained.

‡ Patient 15 was documented as acute poststreptococcal glomerulonephritis, but the renal tissue of this patient was not used in this study.

from each patient were examined, except for Patient 2, where only one glomerulus was found.

In 7 patients (1, 3, 7–11) Fe-IgG-AGN was bound in the mesangial matrix (Figure 8) and, in Patients 7–11, also at the endothelial side of the glomerular basement membranes (Figure 9). Subepithelial deposits did not bind Fe-IgG-AGN (Figure 10).

In the glomeruli of 4 patients (2, 4–6), Fe-IgG-AGN could not be detected, whereas FITC-IgG-AGN stained these patients' glomeruli. In Patients 12–14, in whom the biopsies were obtained between 28 to 32 days, and in the control specimens, neither Fe-IgG-AGN nor FITC-IgG-AGN localized in the glomeruli. Ferritin-conjugated control IgG from normal humans and ferritin-conjugated human albumin were applied to renal tissue from Patients 7 and 8 and were not bound in their glomeruli.

Discussion

Although there is little doubt that glomerular injury in acute poststreptococcal glomerulonephritis is immune complex mediated, it has been difficult to demonstrate the presence of antigenic sites.

This study was undertaken to confirm, by the immunoferritin technic the precise localization and the time of appearance of antigen in glomeruli of patients with acute poststreptococcal glomerulonephritis, as shown previously by the immunofluorescent technic. Labeled IgG fractions from patients with acute poststreptococcal glomerulonephritis were employed for the identification of antigenic sites.

The diagnosis of acute glomerulonephritis in all 14 patients was established by the typical clinical and laboratory findings, including the characteristic glomerular lesions.

Distribution of IgG and C3 in a granular fashion was demonstrated in the glomeruli of all patients. The FITC-labeled IgG of patients with acute glomerulonephritis, however, stained glomeruli only when renal tissue was obtained within 12 days after onset. During the first 7 days numerous fine granular deposits of IgG and C3 were present along the glomerular basement membrane but were not seen in the mesangial region. FITC-IgG-AGN also stained but in a punctate pattern involving parts of basement membranes and mesangium.

After this period, antihuman IgG and C3 staining of the basement membranes became dense and showed fine and coarse deposits. In addition, mesangial staining developed. Deposition of FITC-IgG-AGN decreased or was absent. In the later stage (30 to 32 days after onset), granules along the basement membranes became larger; however, their number decreased and mesangial involvement increased. FITC-IgG-AGN did not stain.

This development seems to indicate that, during the early phase of acute poststreptococcal glomerulonephritis, antigenic sites are not fully saturated and are capable of binding FITC-IgG-AGN, whereas later in the course of the disease full saturation has taken place. The disappearance of the immune complex antigen was similar to that described by Dixon *et al* [13,14] in experimental acute serum sickness.

The postulate that antigenic sites are still available during the early phase is supported by the findings of Michael *et al* [6] and Seegal *et al.*[4] These authors obtained best staining results with FITC-labeled rabbit antisera against streptococcal products when renal biopsies were obtained early during the course of the disease.

A correlation between the staining capacity of FITC-IgG-AGN and time elapsed after onset of acute glomerulonephritis was observed. Sera obtained during the first week after onset showed very little staining. Sera obtained during second to fourth weeks showed increased staining capacity and staining capacity finally disappeared after the fifth week. This demonstration of what appears to be rising and falling antibody titers suggests the presence of specific antibody in the patients' serum which binds to an antigen, probably of streptococcal origin, in glomeruli of patients with acute poststreptococcal glomerulonephritis.

Supporting evidence for the presence of specific antigen in these patients' glomeruli is given by the fact that kidney biopsy specimens, obtained from patients with various other renal diseases, as well as normal kidney tissue failed to stain with any of the FITC-IgG-AGN sera. Preabsorption of FITC-IgG-AGN with normal human glomerular basement membrane did not alter the staining capacity,[8] but staining of FITC-IgG-AGN was eliminated after incubation with disrupted nephritogenic streptococci.[15]

Corroborative evidence of these immunofluorescent findings was shown by our results using ferritin-conjugated IgG from the sera of patients with acute glomerulonephritis. Precise location of antigenic sites could then be demonstrated.

Ferritin-conjugated IgG obtained from patients with acute poststreptococcal glomerulonephritis was found only on the endothelial aspect of the glomerular basement membrane and inside the mesangial matrix but not in subepithelial deposits or mesangial cells. Fe-IgG-AGN could only be seen in segmental distribution. A similar distribution of ferritin-labeled antibody to group A type 12 streptococci was also found by Andres *et al.*[5]

In contrast, ferritin-labeled antihuman IgG was distributed diffusely on the subendothelial aspect of the basement membrane, in the basement membrane, in the mesangium and in subepithelial deposits, as also seen by Andres.[5]

These findings indicate that scattered free antigenic sites exist on the endothelial side of the basement membrane and in the mesangial matrix during the early phase of the disease. On the other hand subepithelial deposits seem to represent fully saturated immune aggregates.

The presence of antigen in the mesangial matrix but not in the mesangial cells is at present unexplained.

There was a discrepancy in some cases between immunofluorescent and immunoferritin findings. In 4 patients (2, 4–6), Fe-IgG-AGN could not be detected, whereas FITC-IgG-AGN was present in the glomeruli. This difference may very well be due to differences in technic and may be explained by occasional insufficient ferritin penetration or local accumulations of bound ferritin that were missed during our observations. Penetration of antibodies labeled with ferritin particles is only very superficial. Thus a section taken somewhat below this level may not show the specific label.

The findings with the immunoferritin technic seem to indicate, again, that, in the initial phase, free antigen is located on the endothelial side of the glomerular basement membrane and in the mesangial matrix in a scattered fashion and can penetrate the basement membrane only when joined by antibody and complement, which in turn increases the permeability of the basement membrane. Thus only antigen-antibody-complement complex aggregates can be found subepithelially after penetration through the basement membrane. Here free antigen, which by itself could not penetrate the membrane, can no longer be found.

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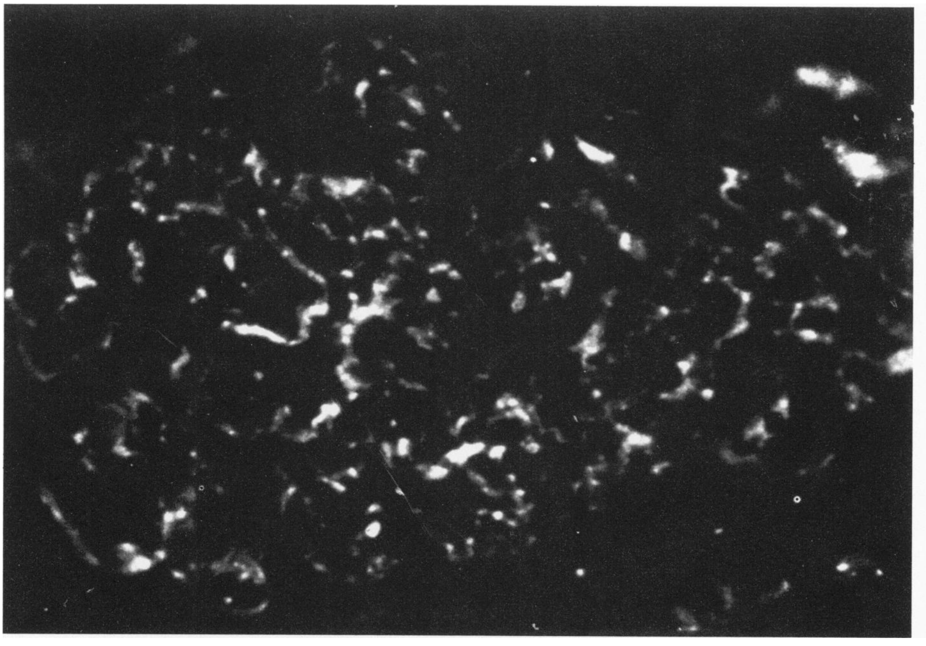

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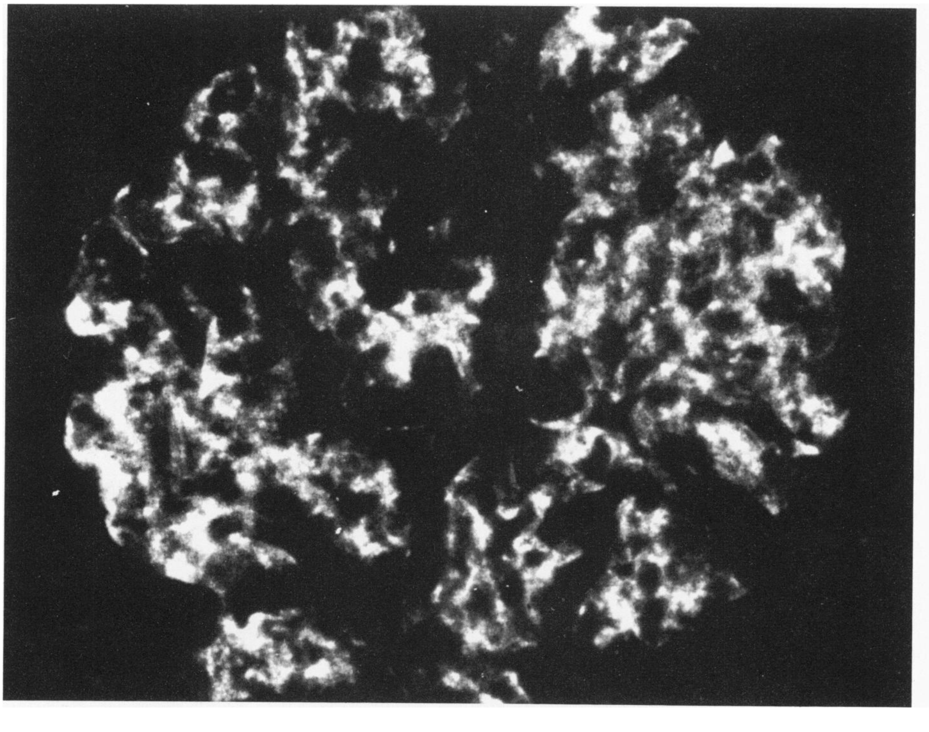

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Fig 1—Patient 2; kidney biopsy 4 days after onset of poststreptococcal acute glomerulonephritis. A glomerulus stained with fluorescein-labeled antihuman C3. Diffuse fine granular deposition of C3 is seen along the basement membrane with no mesangial widening (× 1250). **Fig 2**—Patient 10; kidney biopsy 10 days after onset of acute poststreptococcal glomerulonephritis. A glomerulus stained with fluorescein-labeled antihuman C3. This picture represents diffuse intense staining of the basement membrane with moderate mesangial widening (× 1250).

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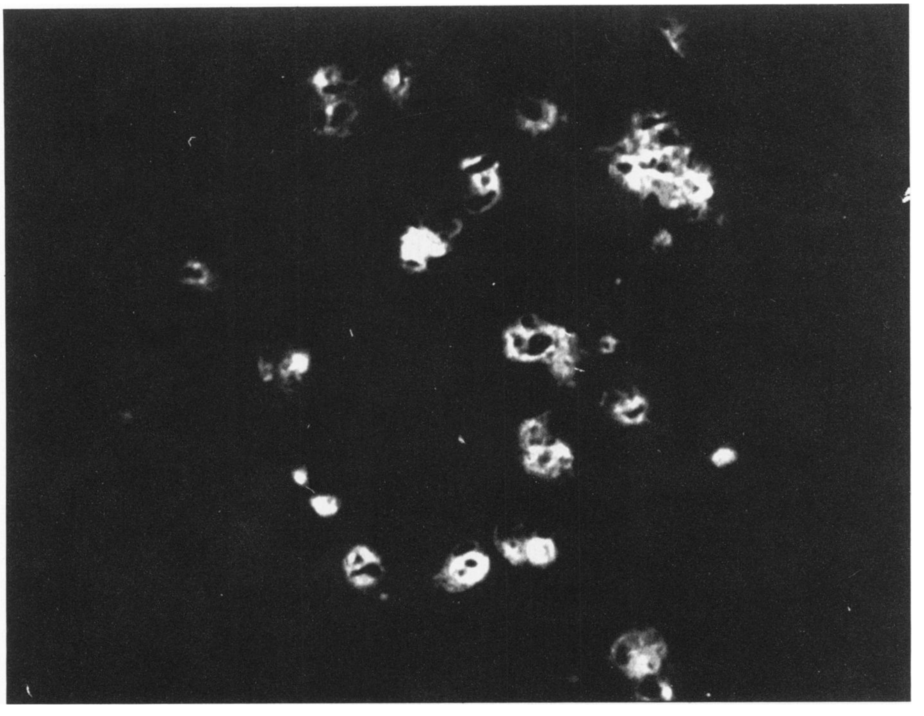

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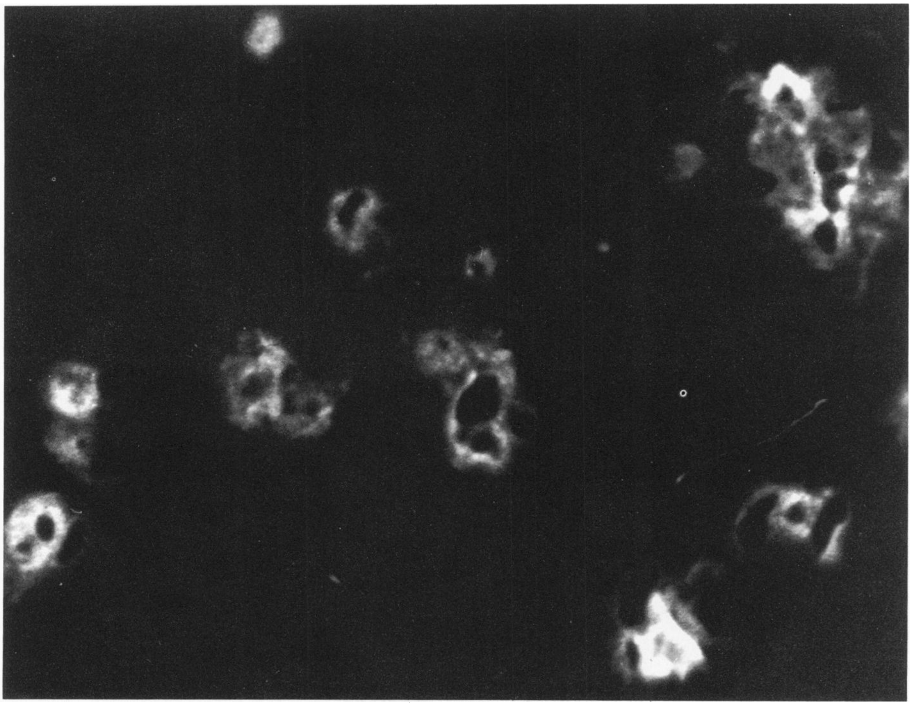

Fig 3—Patient 5; kidney biopsy 5 days after onset of acute poststreptococcal glomerulonephritis. A glomerulus stained with the patient's own fluorescein-labeled IgG. The staining appears ring-like in granular nature (× 1000). **Fig 4**—High power view of the same glomerulus as in Figure 3. Areas of the basement membrane and mesangium are stained with patient's own IgG in ring-like fashion (× 2250).

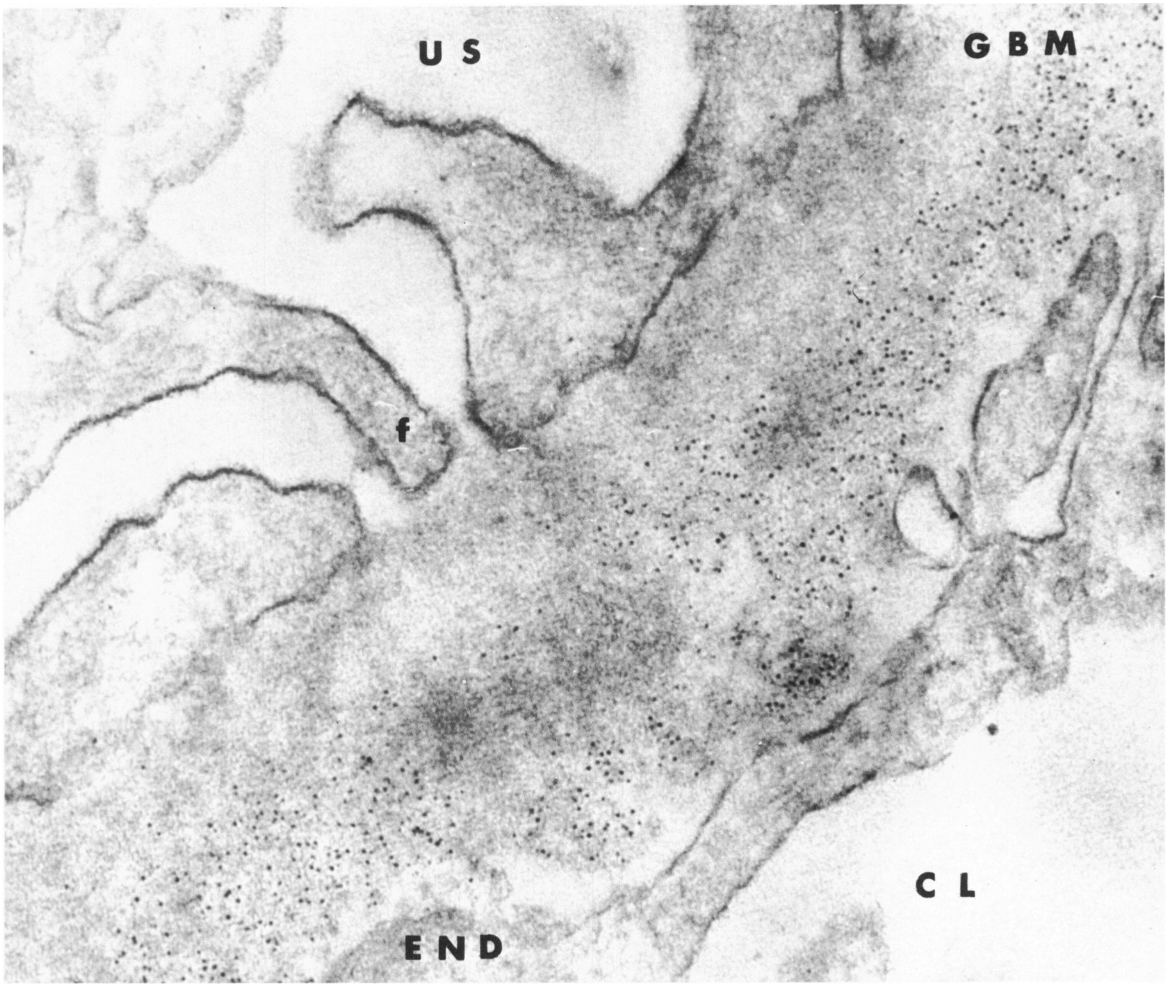

Fig 5—Patient 5; renal tissue treated with ferritin-conjugated antihuman IgG. Ferritin localization on the endothelial side of glomerular basement membrane (*GBM*) *CL* = capillary lumen, *f* = epithelial foot process, *US* = urinary space (× 80,000).

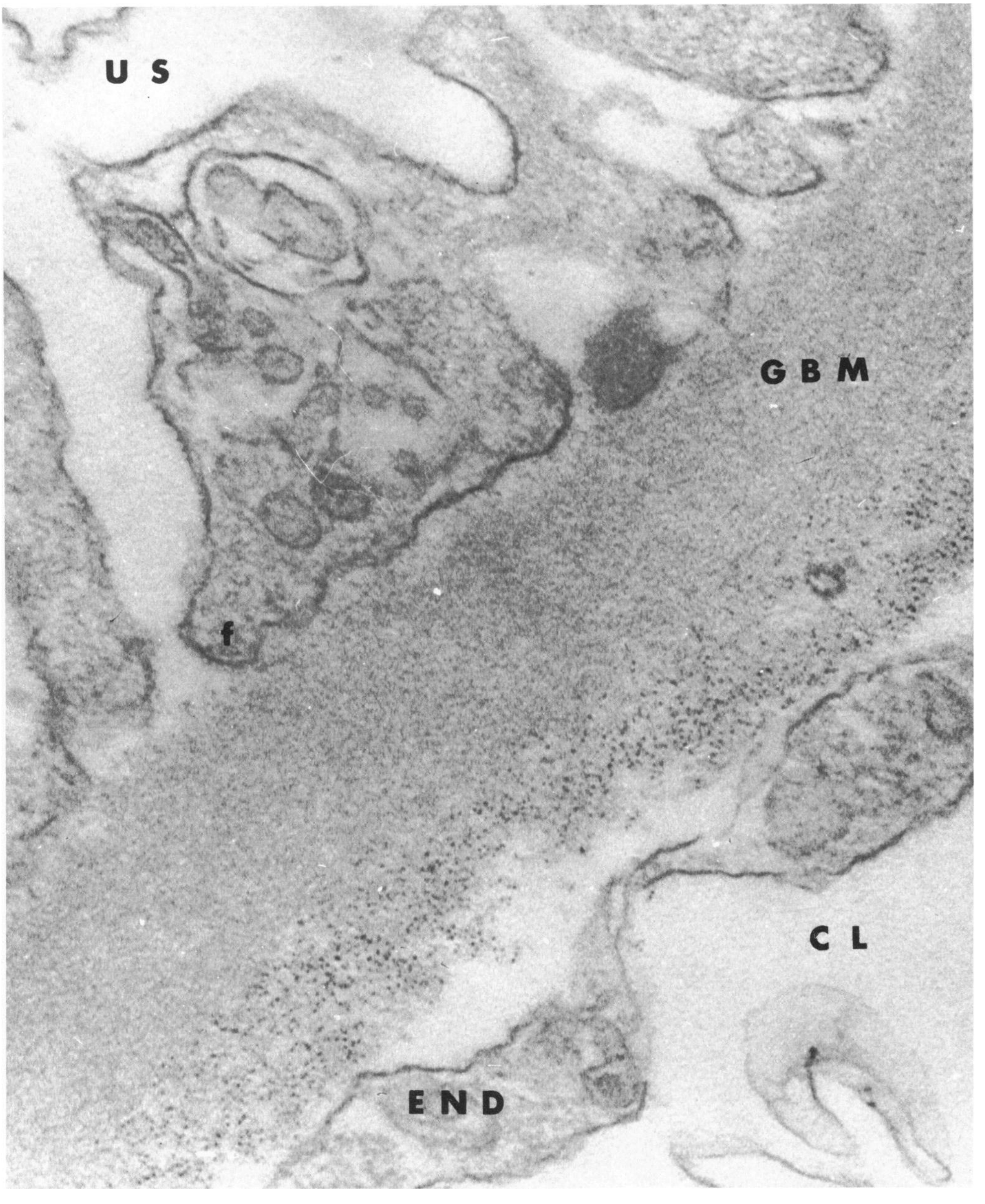

Fig 6—Patient 10; tissue treated with ferritin-conjugated IgG obtained from Patient 2. Ferritin particles are bound on the endothelial side of the glomerular basement membrane (*GBM*). *CL* = capillary lumen, *f* = epithelial foot process, *US* = urinary space (× 80,000).

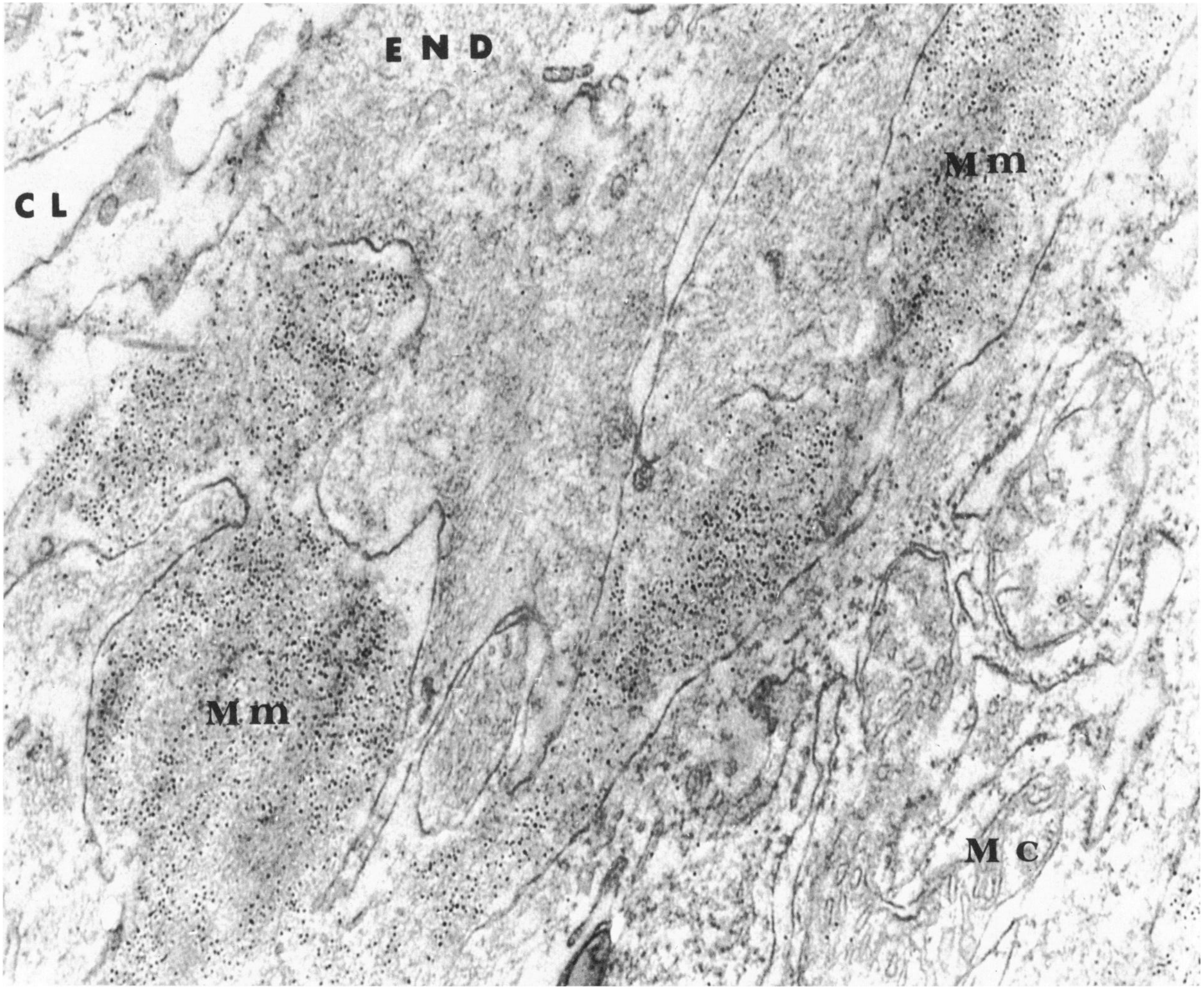

Fig 7—Patient 10; tissue treated with ferritin-conjugated antihuman IgG. Ferritin is localized in the mesangial matrix (*Mm*) lying between the endothelial cell (*END*) and the mesangial cell (*MC*). Capillary lumen (*CL*) is seen in the upper left (× 50,000).

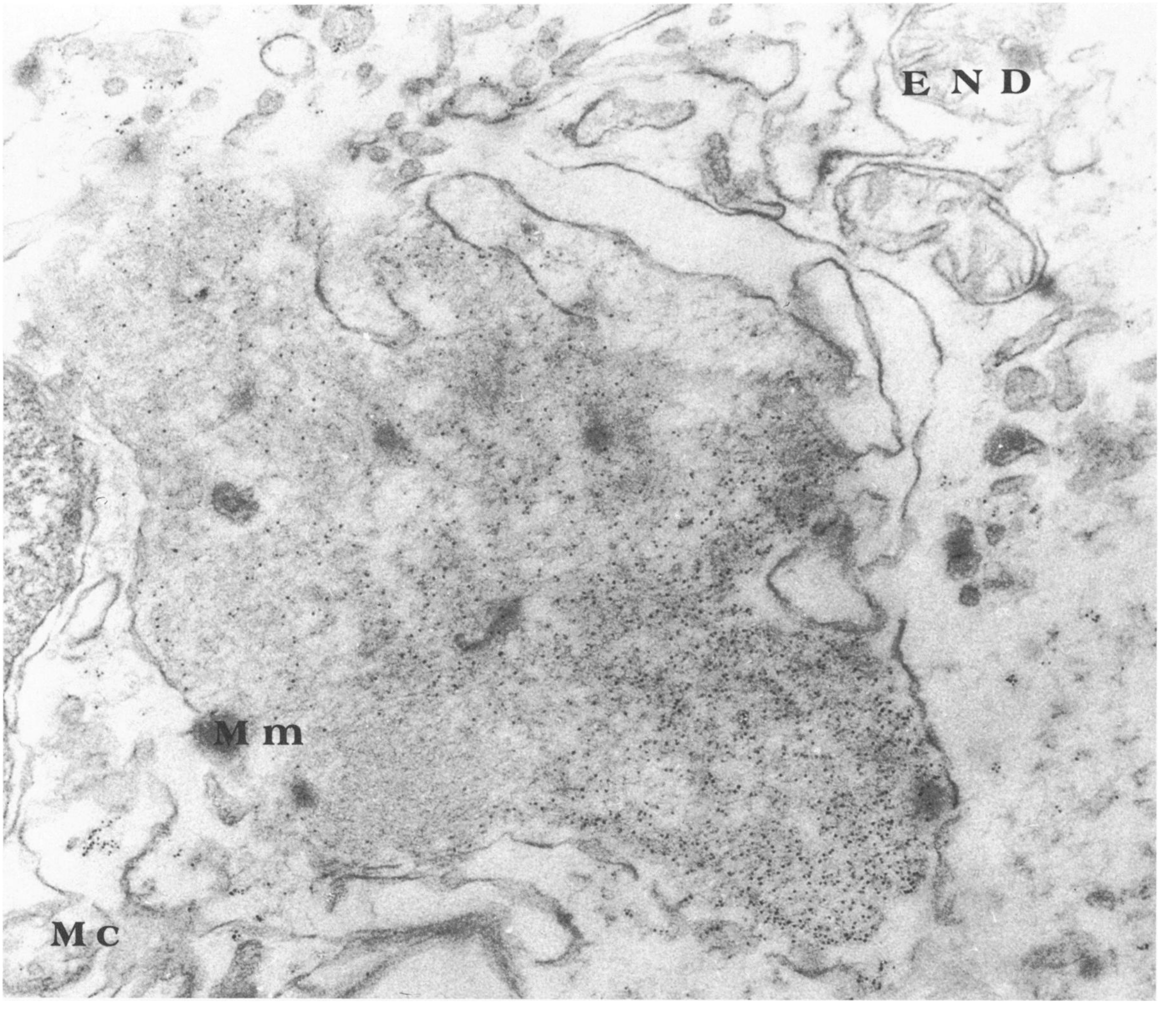

Fig 8—Patient 11; kidney biopsy 12 days after onset of acute poststreptococcal glomerulonephritis. Tissue treated with ferritin-conjugated IgG obtained from Patient 2. Ferritin is specifically bound in the mesangial matrix (*Mm*) but only a few ferritin particles are seen in the mesangial cell (*MC*) and endothelial cell (*END*) (× 50,000).

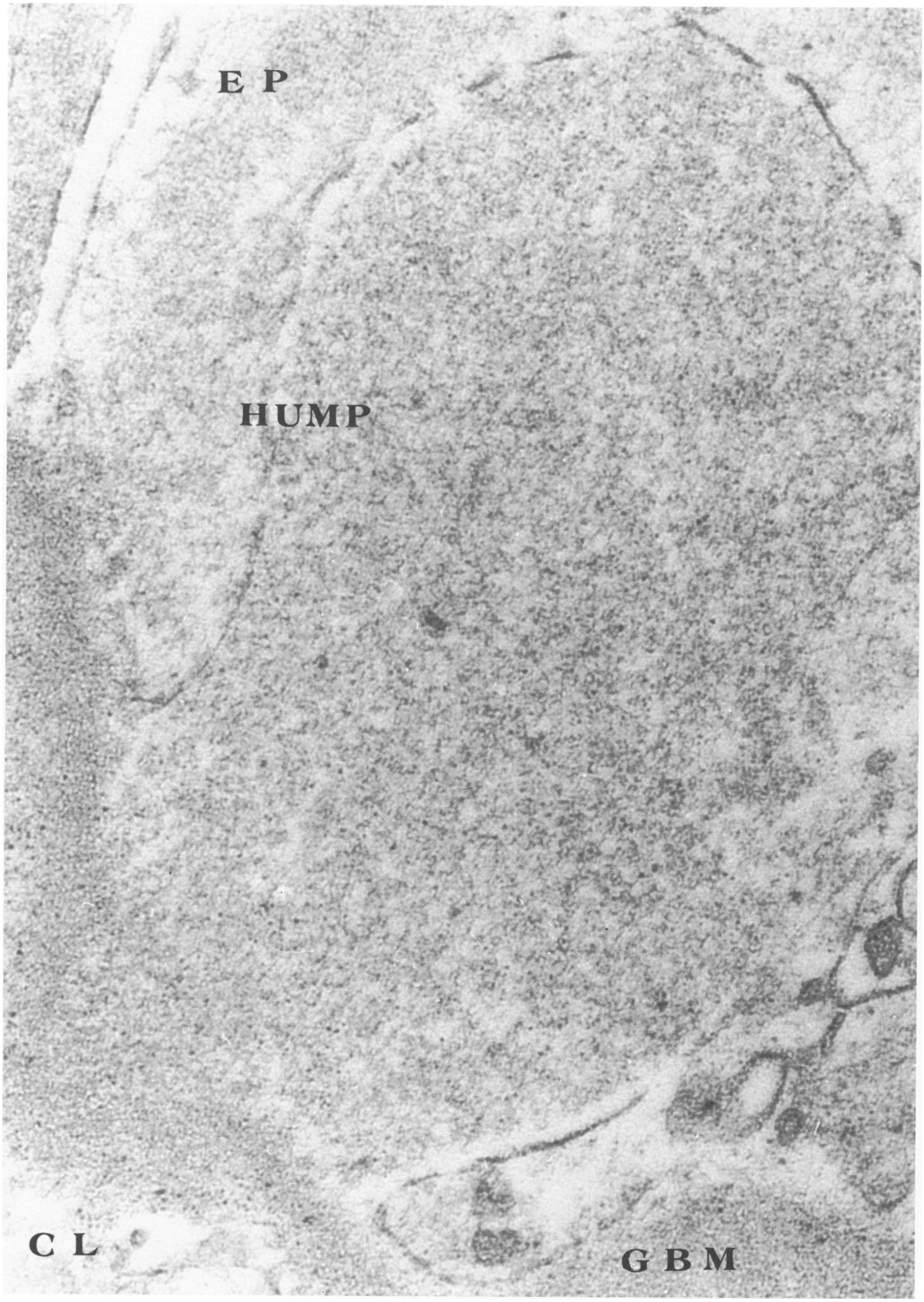

Fig 9—Patient 8; kidney biopsy 7 days after onset of acute poststreptococcal glomerulonephritis. Tissue treated with ferritin-conjugated antihuman IgG. Ferritin is localized in the subepithelial deposit (*HUMP*) and on the endothelial side of glomerular basement membrane (*GBM*). A few ferritin particles are seen in the epithelial cytoplasm (*EP*). *CL* = capillary lumen (× 80,000).

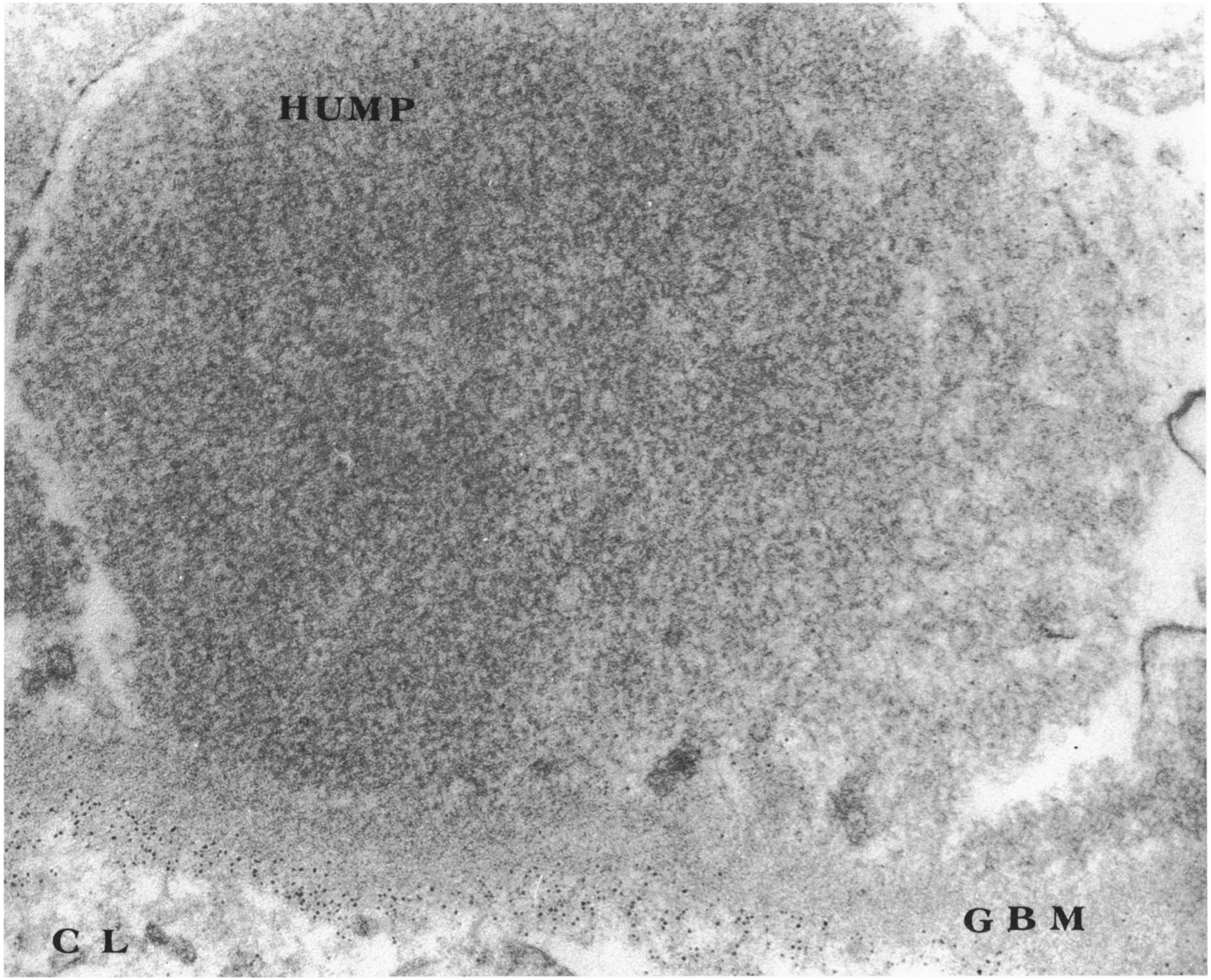

Fig 10—Patient 8; tissue treated with ferritin-conjugated IgG obtained from patient 15. Ferritin is fixed in the endothelial side of glomerular basement membrane (*GBM*) but only a few ferritin particles are seen in the subepithelial deposit (*HUMP*) *CL* = capillary lumen (× 80,000).